their structure. It was also observed that 2',5'-phosphorothioate linkages are more stable than 2',5'-phosphodiester linkages. A plot of the percentage of full length oligonucleotide remaining intact in plasma one hour following administration of an *i.v.* bolus of 5 mg/kg oligonucleotide is shown in Figure 4.

[0201] A plot of the percentage of full length oligonucleotide remaining intact in tissue 24 hours following administration of an *i.v.* bolus of 5 mg/kg oligonucleotide is shown in Figure 5.

[0202] CGE traces of test oligonucleotides and a standard phosphorothioate oligonucleotide in both mouse liver samples and mouse kidney samples after 24 hours are shown in Figure 6. As is evident from these traces, there is a greater amount of intact oliogonucleotide for the oligonucleotides of the invention as compared to the standard seen in panel A. The oligonucleotide shown in panel B included one substituent of the invention at each of the 5' and 3' ends of a phosphorothioate oligonucleotide while the phosphorothioate oligonucleotide seen in panel C included one substituent at the 5' end and two at the 3' end. The oligonucleotide of panel D includes a substituent of the invention incorporated in a 2',5' phosphodiester linkage at both its 5' and 3' ends. While less stable than the oligonucleotide seen in panels B and C, it is more stable than the full phosphorothioate standard oligonucleotide of panel A.

EXAMPLE 57

Control of *c-raf* message in bEND cells using modified oligonucleotides

[0203] In order to assess the activity of some of the oligonucleotides, an in vitro cell culture assay was used that measures the cellular levels of c-raf expression in bEND cells.

Cells and Reagents

[0204] The bEnd.3 cell line, a brain endothelioma, was obtained from Dr. Werner Risau (Max-Planck Institute). Opti-MEM, trypsin-EDTA and DMEM with high glucose were purchased from Gibco-BRL (Grand Island, NY). Dulbecco's PBS was purchased from Irvine Scientific (Irvine, CA). Sterile, 12 well tissue culture plates and Facsflow solution were

purchased from Becton Dickinson (Mansfield, MA). Ultrapure formaldehyde was purchased from Polysciences (Warrington, PA). NAP-5 columns were purchased from Pharmacia (Uppsala, Sweden).

Oligonucleotide Treatment

[0205] Cells were grown to approximately 75 % confluency in 12 well plates with DMEM containing 4.5 g/L glucose and 10 % FBS. Cells were washed 3 times with Opti-MEM pre-warmed to 37 °C. Oligonucleotide were premixed with a cationic lipid (Lipofectin reagent, (GIBCO/BRL) and, serially diluted to desired concentrations and transferred on to washed cells for a 4 hour incubation at 37 °C. Media was then removed and replaced with normal growth media for 24 hours for northern blot analysis of mRNA.

Northern Blot Analysis

[0206] For determination of mRNA levels by Northern blot analysis, total RNA was prepared from cells by the guanidinium isothiocyanate procedure (Monia *et al., Proc. Natl. Acad. Sci. USA,* **1996**, *93*, 15481-15484) 24 h after initiation of oligonucleotide treatment. Total RNA was isolated by centrifugation of the cell lysates over a CsCl cushion. Northern blot analysis, RNA quantitation and normalization to G#PDH mRNA levels were done according to a reported procedure (Dean and McKay, *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 11762-11766). In bEND cells the 2-,5-linked-3'-O-methoxyethyl oligonucleotides showed reduction of *c-raf* message activity as a function of concentration. The fact that these modified oligonucleotides retained activity promises reduced frequency of dosing with these oligonucleotides which also show increased *in vivo* nuclease resistance. All 2',5'-linked oligonucleotides retained the activity of parent 11061 (Table III) oligonucleotide and improved the activity even further. A graph of the effect of the oligonucleotides of the present invention on *c-raf* expression (compared to control) in bEND cells is shown in Figure 7.

EXAMPLE 58

Synthesis of MMI-containing Oligonucleotides

a. Bis-2'-*O*-methyl MMI Building Blocks

[0207] The synthesis of MMI (*i.e.*, R = CH_3) dimer building blocks have been previously described (*see, e.g.*, Swayze, *et al., Synlett* **1997**, 859; Sanghvi, *et al., Nucleosides & Nucleotides* **1997**, *16* 907; Swayze, *et al., Nucleosides & Nucleotides* **1997**, *16*, 971; Dimock, *et al., Nucleosides & Nucleotides* **1997**, *16*, 1629). Generally, 5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyl-3'-C-formyl nucleosides were condensed with 5'-*O*-(N-methylhydroxylamino)-2'-*O*-methyl-3'-*O*-TBDPS nucleosides using 1 equivalent of BH_3 pyridine/1 equivalent of pyridinium para-toluene sulfonate (PPTS) in 3:1 MeOH/THF. The resultant MMI dimer blocks were then deprotected at the lower part of the sugar with 15 equivalents of Et_3N-2HF in THF. Thus the T^*G^{iBu} dimer unit was synthesized and phosphitylated to give T*G(MMI) phosphoramidite. In a similar fashion, A^{BZ}*T(MMI) dimer was synthesized, succinylated and attached to controlled pore glass.

b. Oligonucleotide synthesis

[0208] Oligonucleotides were synthesized on a Perseptive Biosystems Expedite 8901 Nucleic Acid Synthesis System. Multiple 1-μmol syntheses were performed for each oligonucleotide. $A^*_{MMI}T$ solid support was loaded into the column. Trityl groups were removed with trichloroacetic acid (975 μL over one minute) followed by an acetonitrile wash. The oligonucleotide was built using a modified thioate protocol. Standard amidites were delivered (210 μL) over a 3 minute period in this protocol. The $T^*_{MMI}G$ amidite was double coupled using 210 μL over a total of 20 minutes. The amount of oxidizer, 3H-1,2-benzodithiole-3-one-1,1-dioxide (Beaucage reagent, 3.4 g Beaucage reagent/200 mL acetonitrile), was 225 μL (one minute wait step). The unreacted nucleoside was capped with a 50:50 mixture of tetrahyrdofuran/acetic anhydride and tetrahydrofuran/pyridine/1-methyl imidazole. Trityl yields were followed by the trityl monitor during the duration of the synthesis. The final DMT group